IL-23 skin and joint profiling in Psoriatic Arthritis: novel perspectives in understanding clinical responses to IL-23 inhibitors

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ABSTRACT

Objectives

To determine the relationship between synovial versus skin transcriptional/histological profiles in patients with active psoriatic arthritis (PsA) and explore mechanistic links between diseased tissue pathology and clinical outcomes.

Methods

Twenty-seven active PsA patients were enrolled in an observational/open-label study and underwent biopsies of synovium and paired lesional/non-lesional skin before starting anti-TNF (if biologic-naïve) or ustekinumab (if anti-TNF inadequate responders). Molecular analysis of 80-inflammation-related genes and protein levels for IL-23p40/IL-23p19/IL-23R were assessed by real-time-PCR and immunohistochemistry, respectively.

Results

At baseline, all patients had persistent active disease as per inclusion criteria. At primary end-point (16-weeks-post-treatment), skin responses favoured ustekinumab, while joint responses favoured anti-TNF therapies. Principal-component-analysis revealed distinct clustering of synovial tissue gene expression away from the matched-skin. While *IL12B-IL23A-IL23R* were homogeneously expressed in lesional skin, their expression was extremely heterogeneous in paired synovial tissues. Here, IL-23 transcriptomic/protein expression was strongly linked to patients with high-grade synovitis who, however, were not distinguishable by conventional clinimetric measures.

Conclusions

PsA synovial tissue shows a heterogeneous IL-23 axis profile when compared to matched skin. Synovial molecular-pathology may help to identify among clinically indistinguishable patients those with a greater probability of responding to IL-23 inhibitors.

INTRODUCTION

Psoriatic arthritis (PsA) is a chronic heterogeneous inflammatory condition occurring in up to 30% of patients with skin and/or nail psoriasis (PsO), which variably affects the spine, peripheral synovial joints and entheses.[1] Although the mechanisms for such disease heterogeneity are not entirely clear, the interleukin (IL)-23/IL-17 axis is believed to be key in PsO and PsA pathogenesis.[2,3]

IL-23 is a proinflammatory cytokine composed of two subunits (p40, in common with IL-12, and p19, IL-23-specific) and mostly produced by keratinocytes, dendritic and myeloid cells. By binding its cognate receptors (IL-23R/IL-12Rβ1), it stabilises RAR-related-orphan-receptor-gamma-t (RORγt) in T-helper-17 (Th17) cells, which, in turn, release their effector cytokines IL-17, IL-21 and IL-22 to initiate and amplify local autoimmune reactions and chronic inflammation.[2]

Several drugs targeting the IL-23/IL-17 axis have been successfully tested in PsO and PsA.[2] For example, ustekinumab and secukinumab, inhibitors of IL-12/IL-23p40 and IL-17A respectively, are recommended as a second-line biologic treatment for PsA patients inadequate responders to conventional-synthetic (cs) Disease-Modifying-Anti-Rheumatic-Drugs (DMARDs) who had failed at least one Tumour-Necrosis-Factor (TNF) inhibitor (TNFi).[4,5] However, by blocking these pathways, while 47%-64% of patients achieve a 75%-improvement in skin disease [Psoriasis-Area-and-Severity-Index (PASI75)], success in treating joints is more modest, and a mere 20% improvement [American-College-of-Rheumatology (ACR20)] is observed in 35%-50% of patients.[6,7] The new IL-23p19 selective inhibitors have been shown to be more effective, and ACR20 is reached in approximately 60% [8,9]. However, while a similar proportion of patients achieve almost complete psoriasis clearance (PASI90), high hurdles joint disease ACR50/ACR70 is achieved in only 33-36% and 13-20% of patients, respectively.[8,9]

To date, the mechanism for such divergent skin-joint response, consistent across multiple trials, remains largely unexplained. We [2] and others [10] have postulated that different target expression levels in skin and joints contribute to the diverse clinical response. For example, Belasco et al. reported

that gene expression patterns in skin and synovium are distinct, showing a stronger IL-17 signature in skin than in synovium, and more equivalent TNF signal across both tissues [10]. Here we present new evidence exploring the expression of the IL-12/IL-23 axis in psoriatic skin versus matched synovial tissue at both molecular and protein level.

METHODS

Full methods are included in **supplementary material**. Briefly, 27 patients fulfilling the CASPAR criteria [11] with active peripheral joint disease despite csDMARDs and either biologic-naïve/ failing TNFi were recruited in this observational/open-label study (REC15/LO/0584). Patients underwent a baseline ultrasound-guided synovial biopsy [12] and lesional/non-lesional skin punch-biopsies, and were then treated with TNFi/ustekinumab as per local guidelines. The chosen primary endpoint was 16-weeks. Gene expression was analysed by real-time PCR (Fluidigm). Paraffin-embedded skin/synovium samples were stained with Haematoxylin-Eosin. Immune-cells/IL-23-axis were quantified by immunohistochemistry. Synovial tissue were categorised in "low-grade" (score 0-1) or "high-grade" (score 2-7) synovitis [13] and in pathotypes (lympho-myeloid/diffuse-myeloid/pauci-immune) [14].

RESULTS

Patients' characteristics and treatment response

Baseline and 16-weeks demographic and clinical features are summarized in **Figure.1**. The overall male-to-female ratio was ~1:1 (59% female), the average age was 45.4±12.5 and disease duration >10 years. 78% of patients had concomitant skin involvement, with a mean PASI of 7.8. As per inclusion criteria, all patients had active joint disease [68-tender-joints-count 30.9±19.2, 66-swollen-joints-count 13±10.4, Disease-Activity-Score-(DAS) 4.3±1.1] despite treatment with csDMARDs ± anti-TNF. Following the baseline biopsy, patients were treated with anti-TNF (n=18) if they were biologic-naïve or ustekinumab (n=9) if they had not responded to at least one TNFi. The higher number of females in the ustekinumab-arm (8/9) reflects the gender differences in TNFi-treatment outcomes observed in registries [15] (**Figure.1A**). At 16-weeks, ESR, tender-joint scores, Ritchie-Articular-Index (RAI), VAS-

pain, Likert-physician-assessment and DAS were significantly higher in the ustekinumab-treated group; PASI-scores improved from baseline in both groups (-4.7±7.5 in TNFi-treated vs -8.9±14.3 in ustekinumab-treated) and were comparable between the two treatment arms (2.3±2.6 in TNFi-treated vs 2.3±2.3 in ustekinumab-treated) (**Figure.1B**). However, while significantly more patients in the anti-TNF group achieved EULAR(DAS)-response compared with ustekinumab-treated patients (70.6% vs 22.2%), there was a trend in favour of ustekinumab in terms of skin responses (**Figure.1C**). Besides, as joint-response to ustekinumab can be delayed up to 24-28 weeks, clinical responses were also assessed at 24-weeks. As shown in **Supplementary Figure.S1**, ustekinumab-treated patients maintained significantly higher tender joint scores, RAI, VAS-pain, Likert-physician-assessment and DAS-score; 50% and 68.8% of patients in the ustekinumab- and TNFi arms achieved EULAR(DAS)-response, respectively. Individual-patient joint/skin responses are summarized in **Supplementary Table.S1**.

Gene expression profiles in paired skin and synovium reveal tissue-specific signatures and divergent expression patterns

Gene expression analysis was performed on 14 matched synovial tissue, lesional and adjacent non-lesional skin. As shown in **Figure.2A**, principal component analysis (PCA), built on the expression of 80 inflammation-related genes (**Supplementary Table.S2**), showed that the synovium clusters away from the skin, with a partial overlapping of lesional and non-lesional skin. To further investigate the gene variance contributing to the diversity of expression within each anatomic site (skin/synovium), related PCA plots were co-visualised with loading plots (biplots) (**Figure.2B** and **C**). *IL17A/F*, *IL23R* and *IL21* were the major contributors of PC1/2 variation in lesional skin. In synovium, genes related to Ectopic-Lymphoid-Structure (ELS) formation (*CXCL13*, *CXCR5*) and the IL-23 axis (*IL23A*, *IL12B, IL23R*) together strongly contributed to the PC variation. For instance, *CXCR5* and *IL23A* robustly aligned with PC1 in accounting for 35.4% of the variance within the synovium data set and *CXCL13* strongly and equally contributed to PC1 and PC2 variation. We next assessed the relative gene expression of the drug-targets of TNF- and IL-23/IL-12-inhibitors, i.e. *TNF*, *IL23A* (encoding IL-23p19), *IL12B* (encoding IL-23p40) and *IL23R* (**Figure.2D)**. *TNF* was generally homogeneously expressed in both skin and synovial

tissue. Conversely, *IL23A*, *IL12B* and *IL23R* showed higher expression in lesional skin compared to both non-lesional skin and synovium. Interestingly, we observed that while some patients did express IL-23 cytokines/receptor in both skin and joint, others had discordant expression, i.e. active IL-23 pathway in the lesional skin but not in the synovium. To investigate potential mechanisms for the diverse expression of the IL-23-axis within the synovium, we stratified patients based on the degree of synovial inflammation.[13] Both *IL12B* and *IL23R* genes, but not *IL23A*, were significantly more expressed in patients with higher synovitis scores (**Figure.2E**). Notably, despite the major variance in the degree of synovial inflammation and histological pathotypes, there were no significant clinical differences in the two patient-groups (**Supplementary-Table.S3 and S4**).

Synovial IL-23p40/p19 and IL-23R protein expression correlates with the histological inflammatory status

To confirm the molecular findings, we next evaluated protein expression levels of IL-23p40, IL-23p19 and IL-23R in skin and synovium by immunohistochemistry. As expected, the percentage of IL-23p40-, IL-23p19- and IL-23R-positive cells was significantly higher in lesional skin compared to paired non-lesional skin (**Figure.3A and B**); within the synovium, it was greater in patients with higher degree of inflammation (**Figure.3C and D**) and in lympho- and diffuse-myeloid pathotypes (**Supplementary Figure.S2**). This result was in line with the positive correlation observed between the synovial inflammatory score and the proportion of IL-23p40/IL-23p19/IL-23R-positive cells (**Figure 3E**), as well as their correlation with each other's (data not shown). Of note, the percentage of IL-23p40/IL-23p19/IL-23R-positive cells at baseline was, on average, comparable between the treatment groups despite different drug-exposure (**Supplementary Table.S5**). Except for the LIKERT-patient-score, we did not detect other significant correlations between IL-23-axis expression and clinical parameters at baseline, suggesting that patients with comparable disease severity may have, in fact, heterogeneous histopathological features and expression of drug-targets within the diseased synovium (**Supplementary Table.S6**).

To further assess whether the IL-23-axis heterogeneity tracks across different stages of the disease, we analysed IL-23 expression pattern in the synovium of 21 treatment-naïve PsA patients with <12 months symptoms. As shown in **Supplementary Figure**.**S3**, overall, there was a positive correlation between IL-23p40/IL-23p19/IL-23R-positive cells and synovitis scores, and lower IL-23 cytokines/receptor tissue-availability in the pauci-immune compared to macrophage-rich pathotypes. Similarly to established PsA, we did not find significant correlations between clinical parameters and IL-23 axis expression. Finally, to investigate whether the differential IL-23-expression observed in PsA synovium was disease-specific or related to synovial histopathology, we quantified IL-23p40/IL-23p19/IL-23R in a cohort of 17 treatment-naïve rheumatoid arthritis (RA) patients spanning diverse degrees of synovial inflammation and histopathotypes, and confirmed that, at least in the early phases of RA, IL-23 expression pattern is pathology-related and significantly associates with the presence of ELS (**Supplementary Figure.S4**).

DISCUSSION

To our knowledge, this study provides first-time detailed evidence of the expression of the IL-23 axis (IL-23p40/ IL-23p40p19/IL-23R), both at transcript and protein level, in matched skin-synovium obtained from clinically active PsA patients before undergoing anti-TNF or ustekinumab.

Using a PCR-Fluidigm-assay of 80 inflammation-related genes, first, we demonstrated distinct synovial gene expression clustering away from paired skin but a partial overlapping between lesional and non-lesional skin profiles. We also showed that IL-17 and IL-23 cytokines together with CXCL13/CXCR5, key chemokines involved in ELS formation, significantly contribute to the gene expression variance within skin and joint sites, respectively. These results are in line with those reported by Belasco et al. [10] demonstrating that IL-17 is a major contributor of the gene expression variability within the lesional skin, and Celis et al. [16] who showed that in synovial biopsies (unmatched for skin samples) the expression of IL-23 correlates with ELS-positive samples.

The analysis of the expression profiles of biologic-DMARDs-targets demonstrated that *TNF* was more homogeneously expressed in skin and synovial tissue, while *IL23A*/*IL12B*/*IL23R* were generally higher-expressed in lesional skin compared to both non-lesional skin and synovium. The synovial expression of *IL23A/IL12B/IL23R* was, in fact, greatly heterogeneous and could be either similar to or much lower than the paired lesional skin. Notably, *IL12B* and *IL23R* transcripts levels were dependent on the degree of tissue inflammation, being more expressed in the presence of higher synovitis scores. Similarly, we confirmed a preferential expression of IL-23p40/IL-23p19/IL-23R proteins in patients with high-grade synovitis and immune-cells-rich histopathotypes. Importantly, patients with variable degrees of synovial inflammation and diverse pathotypes, as well as different levels of IL-23-cytokines/receptor could not be phenotypically distinguished by conventional clinical scores. Furthermore, despite variable drug-exposure, the pathology of the IL-23 axis in active patients was comparable at baseline. We confirmed that IL-23-axis expression relates to the synovial histopathology not only in PsA at different stages of the disease, including early treatment-naïve patients, but also in the early phase of RA, investigated as disease control. Therefore, the pattern of expression of the IL-23 axis does not seem to be disease-specific but rather dependent on the inflammatory status and histological features of the synovial tissue in both PsA and RA.

While it is generally accepted that patients with high disease activity respond better to biologics, clinimetric measures cannot determine the grade of histological synovitis or drug-target expression levels. Tissue bioavailability of the “target”, of course, does not guarantee clinical response; however, there is evidence to suggest that, for example, TNF levels in RA synovium are associated with better response to TNFi, [17] and other specific synovial tissue signatures are linked with different outcomes to anti-TNF [18] and anti-IL-6R therapy.[19] The results reported here support the concept that heterogeneous drug-target bioavailability in the diseased tissue might also apply to the IL-23 axis. This prompted the hypothesis that different joint response rates in PsA, often divergent from the skin-response, might be explained, at least partially, by the preferential expression of the IL-23-axis by subsets of patients with higher histological synovitis but not necessarily higher disease activity.

PsABRE was an exploratory study, not designed to assess efficacy; thus, the relatively small sample-size in each treatment-arm did not allow to test the above hypothesis. Moreover, no direct comparisons could be carried out between the anti-TNF- and the ustekinumab-treated cohorts: both populations failed to respond to csDMARDs, but whilst the former was biologic-naïve, the latter had inadequately responded to at least one TNFi representing, therefore, a more difficult-to-treat group. The trial took place in a real-life setting with no external or industry support; hence, the recruitment and treatment allocation had to follow the UK National Institute for health and Care Excellence (NICE) prescription-guidelines with consequent different drug-exposure in the two groups. Despite these limitations, the main value of the study resides in its molecular pathology characterization of paired skin and US-guided synovial biopsies of the most inflamed joint, including small joints, that demonstrates a divergent profile between the two diseased tissues and, generally, a lower level of expression of the IL-23 axis in the synovial tissue particularly in patients with low-grade synovitis.

The heterogenous synovial expression of the IL-23-axis provides a plausible mechanistic explanation for the divergent outcomes consistently observed in clinical trials whereby IL-23i have better results in PsA skin than in joints. This hypothesis needs to be tested in larger, appropriately designed and powered studies. Identifying biomarkers of joint-response to therapy in patients clinically indistinguishable is going to be vital to refine PsA clinical classification and enrich for treatment response while reducing unnecessary exposure to costly and potentially toxic medications.

KEY MESSAGES

What is already known about this subject?

• PsA is a chronic heterogeneous inflammatory condition affecting patients with psoriasis, and the IL-23/IL-17 axis is believed to be key in psoriasis and PsA pathogenesis.

• Several drugs targeting the IL-23/IL-17 axis have been successfully tested in the context of psoriasis and PsA but, while 50-60% of patients achieve almost complete psoriasis clearance upon treatment,

the joint disease improvement is modest. To date, the mechanism for the divergent skin-joint response remains largely unexplained.

What does this study add?

• It provides first-time detailed evidence of the expression of the IL-23 axis in matched skin and synovial tissue from active PsA patients demonstrating distinct gene expression clustering of the synovium away from paired skin. It reveals that, while *IL23A, IL12B* and *IL23R* are expressed at a high level in lesional skin, their expression in the synovium is hugely heterogeneous.

• It demonstrates that, while patients with diverse degrees of synovial inflammation could not be distinguished clinically by conventional clinimetric measures, the IL-23 axis signature is differentially expressed within the synovial tissue and strongly linked to high-grade synovitis.

How might this impact on clinical practice or future developments?

• This study demonstrates that PsA synovial tissue shows a heterogeneous IL-23 axis profile independently of its expression in paired-skin samples, thus providing a plausible mechanistic explanation for the divergent skin and joint clinical response to IL-23 inhibitors. It supports the need to test in larger appropriately designed and powered studies whether drug-target bioavailability correlates with the likelihood of response. Identifying biomarkers of joint-response to therapy in patients clinically indistinguishable is going to be vital to improve disease outcomes, prevent disability and reduce healthcare and societal costs.

COMPETING INTEREST:

None declared

AUTHORS CONTRIBUTION

All authors have contributed some critical components to enable the delivery of the study and manuscript. These include: patient recruitment and/or data generation and/or analysis as well as

writing or critically revising the present manuscript and/or raising funds and infrastructure to support the study.

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FIGURE CAPTIONS

Figure 1 Baseline and 16-weeks characteristics of the patients included in the Psoriatic Arthritis Pathobiology and its Relationship with Clinical Disease Activity (PsABRE) study. A, Baseline features of the whole cohort (n=27) and comparison of variables between patients receiving anti-TNF (n=18) or ustekinumab (n=9). B, Patients' characteristics at the chosen primary endpoint, i.e. 16-weeks post-treatment (n=26, one patient lost to follow-up) and comparison between TNFi- (n=17) and ustekinumab-treated patients (n=9). A-B, p-values calculated using Mann–Whitney U-test or Fisher's exact test as required (TNFi-arm vs ustekinumab-arm). C, Skin (PASI50) and joints [EULAR(DAS) good/moderate vs none] response at 16-weeks. p-values calculated using Fisher's exact test. SD, Standard Deviation; n, number; ESR, erythrocyte sedimentation rate; CRP, C-Reactive Protein; TJC, Tender Joints Count; SJC, Swollen Joints Count; RAI, Ritchie Articular Index; PASI, Psoriasis Area and Severity Index; VAS, Visual Analogue Scale (0-100); GH, Global Health; DAS, Disease Activity Score; HAQ, Health Assessment Questionnaire; TNF, Tumour Necrosis Factor; DMARDs, Disease Modifying Anti-Rheumatic Drugs; ns, non-significant; EULAR, European League Against Rheumatism.

Figure 2 Gene expression analysis in matched skin and synovium from PsA patients. A, Principal component (PC) analysis performed on the expression data of a set of 80 selected genes in 14 matched non-lesional (non les.) and lesional skin and synovium. The first two eigenvalues were plotted with data ellipses for each tissue type using a confidence interval of 0.95. The PCA clearly separates synovium (blue dots) from non-lesional skin (non les., green dots) and lesional skin (red dots). B-C, Biplots showing individuals repartition in PC1 and 2 (black dots) and loading plots assessing the contribution of each of the 80 genes analysed in the PC, displayed for the lesional skin (B) and synovial tissues (C). Genes names are indicated if their contribution to the PC variance is >1. D, Heatmap representing *TNF*, *IL12B* (IL-23p40 protein), *IL23A* (IL-23p19 protein) and *IL23R* expression in 14 matched non-lesional (non les.) and lesional skin and synovium samples. dd-threshold cycle (ddCT) are shown in colorimetric scale (low expression in blue, high expression in red). Lines 1 to 11 represent anti-TNF-treated patients, lines 12 to 14 ustekinumab-treated patients. E, I*L12B*, *IL23A* and *IL23R* gene

expression in synovial biopsies classified as “low” (0-1) and “high” (2-7) synovial inflammatory score (Krenn’s score). p-values were calculated using Mann–Whitney U-test, * = $p < 0.05$, mean and standard deviation are shown.

Figure 3 Expression of IL-23p40, IL-23p19 and IL-23R in skin and synovium from PsA patients. A and C, Representative images of sections of PsA non-lesional (non les.) and lesional skin (A), and synovial tissue of different degree of inflammatory scores (C) immuno-stained for IL-23p40, IL-23p19 and IL-23R. Scale bar = 200µm. Enlarged images correspond to the respective boxed areas. B and D, Digital image analysis was performed on non-lesional and lesional skin (B) (n=11-12) and synovium (D) (low inflammatory score, n=4-8; high inflammatory score, n=13-14) sections. IL-23p40, IL23p19 and IL23R positive cells were determined using QuPath software and are presented as % of the total number of cells. Results are shown as mean ± standard deviation. * = $p < 0.05$, **= $p < 0.01$ as assessed by Mann–Whitney U-test. E, Correlations between inflammatory scores and IL-23p40, IL-23p19 or IL-23R percentages of positive cells within the synovial tissue. p-values, calculated by Spearman’s bivariate correlation analysis, are indicated on each graph.

Figure 1

A

		TOTAL POPULATION (27 PATIENTS)	ANTI-TNF (18 PATIENTS)	USTEKINUMAB (9 PATIENTS)	p-values
BASELINE	Female % (n)	59% (16)	44.4% (8)	88.9% (8)	p=0.03
	Age years (mean ± SD)	45.4 ± 12.5	42.2 ± 11.2	51.8 ± 12.9	ns
	Disease duration years (mean ± SD)	10.2 ± 11.8	7.3 ± 7.2	15.3 ± 16.6	ns
	ESR mm/hr (mean ± SD)	24 ± 16.7	22.8 ± 15.4	26.3 ± 19.8	ns
	CRP mg/l mean (mean ± SD)	11.5 ± 21.1	11.4 ± 22.7	11.7 ± 18.6	ns
	TJC/78 (mean ± SD)	34.2 ± 22.6	31 ± 25.1	40.7 ± 15.9	ns
	TJC/68 (mean ± SD)	30.9 ± 19.2	27.7 ± 21.2	37.2 ± 13.1	ns
	RAI (mean ± SD)	18.7 ± 9.8	16.6 ± 10.7	23.1 ± 5.9	ns
	SJC/76 (mean ± SD)	13.6 ± 10.8	11.6 ± 8.5	17.7 ± 13.9	ns
	SJC/66 (mean ± SD)	13 ± 10.4	11.3 ± 8.6	16.3 ± 13.1	ns
	SJC/44 (mean ± SD)	10.3 ± 8.3	9.2 ± 7.8	12.6 ± 9.3	ns
	Skin involvment, yes % (n)	78% (21)	83,3% (15)	66.7% (6)	ns
	PASI (mean ± SD)	7.8 ± 10.7	6.5 ± 8.1	11 ± 16	ns
	VAS tiredness (mean ± SD)	60 ± 30.2	59.1 ± 30.4	61.8 ± 31.6	ns
	VAS pain (mean ± SD)	70.6 ± 21.7	70.3 ± 17.1	71.2 ± 30.2	ns
	VAS GH Patient (mean ± SD)	70 ± 25.1	72.3 ± 20.8	65.4 ± 33.1	ns
	VAS GH Physician (mean ± SD)	62.2 ± 16.2	61.2 ± 16.4	64.2 ± 16.5	ns
	LIKERT Patient (mean ± SD)	3.8 ± 0.8	3.8 ± 0.8	3.9 ± 0.9	ns
	LIKERT Physician (mean ± SD)	3.6 ± 0.6	3.4 ± 0.6	3.8 ± 0.7	ns
	DAS (mean ± SD)	4.3 ± 1.1	4.1 ± 1.2	4.8 ± 1	ns
	HAQ (mean ± SD)	1.9 ± 0.6	1.8 ± 0.6	2.1 ± 0.5	ns
	Previous anti-TNF, yes % (n)	33.3% (9)	0% (0)	100% (9)	p<0.0001
	Current DMARDs, yes % (n)	70.4% (19)	66.7% (12)	77.8% (7)	ns

B

		TOTAL POPULATION (26 PATIENTS)	ANTI-TNF (17 PATIENTS)	USTEKINUMAB (9 PATIENTS)	p-values
16 WEEKS	ESR mm/hr (mean ± SD)	17.6 ± 14.6	12.2 ± 8.8	27.7 ± 18.4	p=0.05
	CRP mg/l mean (mean ± SD)	6.3 ± 8.7	4.2 ± 6.7	10.4 ± 10.8	ns
	TJC/78 (mean ± SD)	25.7 ± 22.1	28.2 ± 20.9	39.7 ± 17.9	p=0.01
	TJC/68 (mean ± SD)	23.3 ± 19.5	16.1 ± 18.0	36.8 ± 15.0	p=0.007
	RAI (mean ± SD)	13.9 ± 10.6	10.9 ± 11.1	19.4 ± 7.2	p=0.02
	SJC/76 (mean ± SD)	8.3 ± 9.3	7.4 ± 8.0	10.1 ± 11.8	ns
	SJC/66 (mean ± SD)	7.8 ± 9.0	7.0 ± 8.0	9.4 ± 11.0	ns
	SJC/44 (mean ± SD)	5.8 ± 6.2	5.6 ± 6.0	6.3 ± 6.9	ns
	PASI (mean ± SD)	2.2 ± 2.5	2.3 ± 2.6	2.3 ± 2.3	ns
	VAS tiredness (mean ± SD)	54 ± 31.6	50.1 ± 31.9	61.3 ± 31.6	ns
	VAS pain (mean ± SD)	46.7 ± 32.2	37.2 ± 30.2	64.6 ± 29.4	p=0.05
	VAS GH Patient (mean ± SD)	50.2 ± 33.4	46.5 ± 33.9	57.1 ± 33.3	ns
	VAS GH Physician (mean ± SD)	45.0 ± 26.0	38.8 ± 24.3	56.7 ± 26.1	ns
	LIKERT Patient (mean ± SD)	2.9 ± 1.1	2.8 ± 1.1	3.1 ± 1.2	ns
	LIKERT Physician (mean ± SD)	2.9 ± 1.3	2.5 ± 1.2	3.8 ± 1.1	p=0.01
	DAS (mean ± SD)	3.4 ± 1.4	3.0 ± 1.5	4.1 ± 0.9	p=0.03
	HAQ (mean ± SD)	1.5 ± 0.9	1.2 ± 1.0	2.0 ± 0.7	ns

C

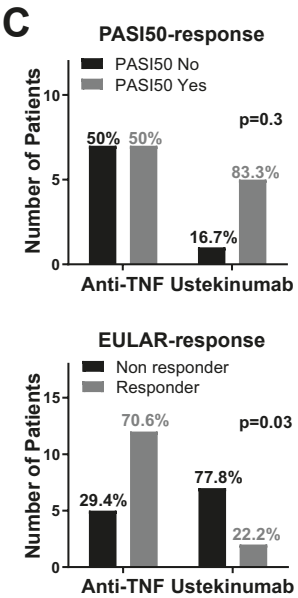
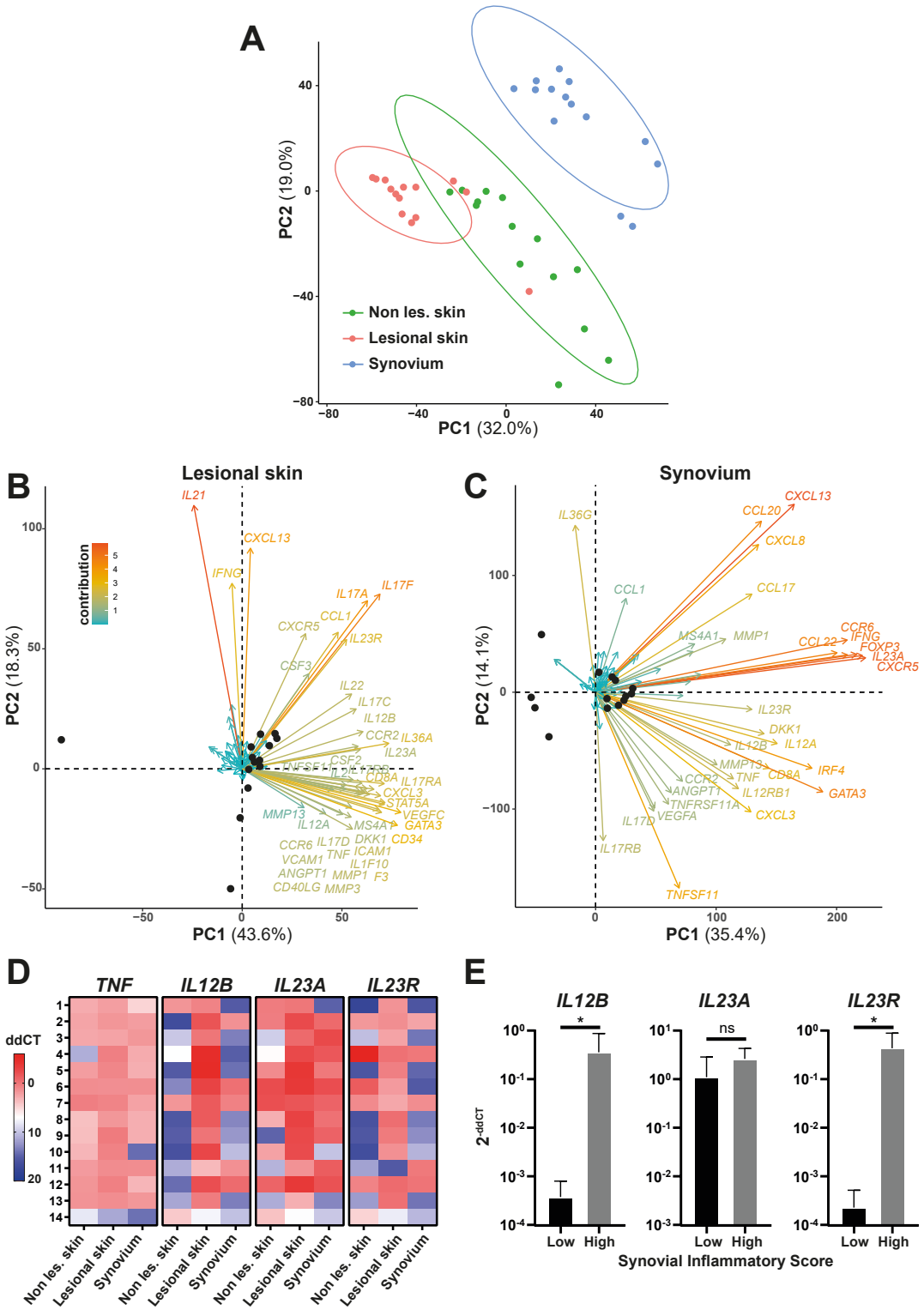
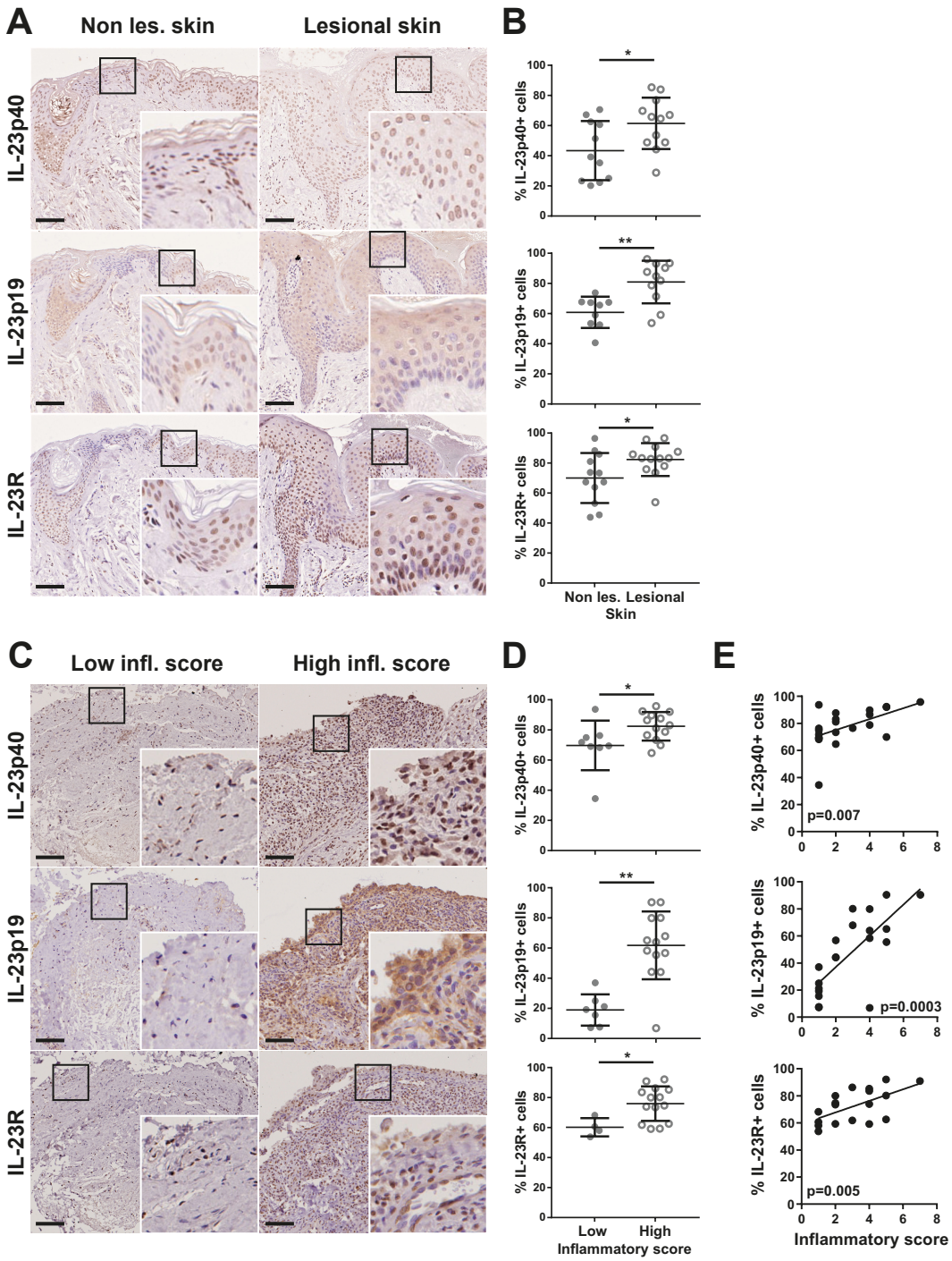


Figure 2


A
PC2 (19.0%)
PC1 (32.0%)
Non les. skin
Lesional skin
Synovium
B
Lesional skin
contribution
PC2 (18.3%)
PC1 (43.6%)
IL21
CXCL13
IFNG
IL17A
IL17F
CCL1
CXCR5
IL23R
CSF3
IL22
IL17C
IL12B
CCR2
IL36A
IL23A
CSF2
CD8A
IL17RA
CXCL3
STAT5A
VEGFC
GATA3
MMP13
IL12A
MS4A1
CCR6
IL17D
DKK1
CD34
TNF
ICAM1
VCAM1
IL1F10
ANGPT1
MMP1
F3
CD40LG
MMP3
C
Synovium
PC2 (14.1%)
PC1 (35.4%)
IL36G
CXCL13
CCL20
CXCL8
CCL1
CCL17
MS4A1
MMP1
CCR6
CCL22
IFNG
FOXP3
IL23A
CXCR5
IL23R
DKK1
IL12B
IL12A
MMP13
IRF4
CCR2
TNF
CD8A
GATA3
ANGPT1
IL12RB1
TNFRSF11A
CXCL3
IL17D
VEGFA
IL17RB
TNFSF11
D
TNF
IL12B
IL23A
IL23R
ddCT
Non les. skin
Lesional skin
Synovium
E
IL12B
IL23A
IL23R
2-ddCT
*
ns
*
Low
High
Synovial Inflammatory Score

1

Figure 3


A
Non les. skin
Lesional skin
IL-23p40
IL-23p19
IL-23R
B
% IL-23p40+ cells
% IL-23p19+ cells
% IL-23R+ cells
Non les. Lesional Skin
C
Low infl. score
High infl. score
D
Low High
Inflammatory score
E
p=0.007
p=0.0003
p=0.005
Inflammatory score

SUPPLEMENTARY METHODS

Patients

Twenty-seven (27) patients fulfilling the ClASsification for Psoriatic ARthritis (CASPAR) criteria [11] with active peripheral joint disease (≥3 tender and ≥3 swollen joints) despite an adequate trial of at least two csDMARDs and either biologic-naïve or failing one or more TNFα-inhibitor (TNFi) were recruited to this observational/open-label real-life study **Ps**oriatic **A**rthritis Patho**B**iology and its **R**elationship with Clinical Diseas**E** Activity (PsABRE) at Bart's Health NHS Trust. All patients underwent a baseline ultrasound (US)-guided needle synovial biopsy of the most inflamed peripheral joint, including small joints, as previously described.[12] Lesional and adjacent non-lesional skin punch biopsies were collected from patients with active skin disease at baseline. According to the National Institute for Health and Care Excellence (NICE UK) prescribing guidelines, patients biologic-naïve were treated with TNFi while patients who had already failed one or more anti-TNF agents received ustekinumab. The chosen primary endpoint was 16-weeks. Clinical and response data were also collected at 24-weeks in view of the delayed response to ustekinumab. Summary of treatments and associated EULAR/PASI50 responses at 16 and 24-weeks are presented in **Supplementary Table.S1**. Synovial/skin biopsy samples were partly stored in RNA-later and partly fixed in formalin. All patients gave written informed consent prior to recruitment. The study was approved by the local ethics committee (REC 15/LO/0584).

To validate the relationship between the IL-23-axis expression and the synovial histopathology, 21 psoriatic arthritis and 17 rheumatoid arthritis patients part of the Pathobiology of Early Arthritis Cohort (05/Q070/198) were also analysed. All patients had <12 months duration of symptoms and were treatment-naïve when underwent US-guided synovial biopsy.

Patient and Public Involvement statement

No funding or time were specifically allocated to patient and public involvement (PPI) in the original application. However, a patients' survey was conducted at the time of the trial to ensure patients were satisfied with the care received as part of the research study and to receive feedback to improve the delivery of the study.

Gene expression analysis

Total RNA was extracted from synovium and skin using a Trizol/Chloroform method as previously described.[14] The relative expression of 80 genes relevant to inflammatory pathways, including genes encoding IL-23-cytokines/receptor, was quantified by real-time PCR using Fluidigm technology (Fluidigm corporation, San Francisco, CA-USA) (**Supplementary Table.S2**). Data were analysed with a

ΔΔCt method using beta-glucuronidase (GUSB) as the housekeeping gene and a mix of cDNA from 3 synovial-tissue samples as reference.

Histology, immunohistochemistry and image analysis

Skin specimens (lesional/non-lesional) and a minimum of 6 synovial-tissue fragments were paraffin-embedded and sectioned at 3µm. Synovial samples were stained with Haematoxylin and Eosin (H&E), and the presence/degree of synovitis was quantified based on parameters previously published [13] by two independent observers; samples were defined as "low-grade synovitis" if score 0-1 or "high-grade synovitis" if score 2-7. Moreover, as previously described in RA synovial tissue [14], synovial tissue sections were immuno-stained for CD3, CD20, CD68 and CD138 in order to quantify the immune infiltrate of T-cells, B-cells, macrophages and plasma cells, respectively. Based on a combination of semi-quantitative score [14], patients were categorised in histological pathotypes (lympho-myeloid, diffuse-myeloid and fibroid/pauci-immune) as previously described [14]. To assess the presence and distribution of IL-23-related cytokines and receptors-(R), both synovial and skin specimens were immuno-stained for IL-23p40 (Novus Biologicals, Centennial, CO-USA), IL-23p19 (BioLegend, San Diego, CA-USA) and IL-23R (Novus Biologicals). Matching isotype antibodies were used as controls. IL-23R cell specificity has also been tested by double immunofluorescence staining with CD3, CD68 and CD20 (data not shown). Slides were counterstained with Haematoxylin and mounted with DPX mounting medium (Sigma-Aldrich, Saint-Louis, MO-USA). All sections were scanned using the digital slide scanner Nanozoomer S210 (Hamamatsu Photonics, Japan). The percentage of positive cells was determined by quantitative digital image analyses using QuPath software.[20]

Statistical analysis

Differences in continuous variables were analysed by Mann–Whitney U-test (two groups) or Kruskal–Wallis with Dunn's post-test (multiple groups). Fisher's exact test was used to evaluate significant associations between categorical variables. Correlations were analysed by Spearman's correlation test. Statistical analyses were performed using GraphPad-Prism-v8-software, p-values<0.05 were considered significant. The PCA and biplots were created using function prcomp from the stats package within R statistics (version 3.5.3) and factoextra R package.[21] The first two eigenvalues were plotted with data ellipses for each tissue type using a confidence interval of 0.95.

Supplementary Figure S1

A

		TOTAL POPULATION (n=24)	ANTI-TNF (n=16)	USTEKINUMAB (n=8)	p-values
24 WEEKS	ESR mm/hr (mean ± SD)	14.8 ± 13.0	11.6 ± 10.0	21.3 ± 16.4	ns
	CRP mg/l mean (mean ± SD)	4.8 ± 7.7	3.8 ± 6.3	7.0 ± 10.1	ns
	TJC/78 (mean ± SD)	23.2 ± 22.6	17.6 ± 23.0	34.5 ± 18.1	p=0.03
	TJC/68 (mean ± SD)	21.0 ± 19.6	16.1 ± 20.0	30.9 ± 15.3	p=0.03
	RAI (mean ± SD)	11.8 ± 10.1	8.4 ± 8.9	18.6 ± 9.4	p=0.01
	SJC/76 (mean ± SD)	6.2 ± 8.4	4.5 ± 7.4	9.5 ± 9.9	ns
	SJC/66 (mean ± SD)	5.5 ± 7.7	4.1 ± 7.2	8.5 ± 8.2	ns
	SJC/44 (mean ± SD)	4.1 ± 5.4	3.3 ± 5.6	5.9 ± 4.9	ns
	PASI (mean ± SD)	2.0 ± 2.6	2.2 ± 2.7	1.6 ± 2.8	ns
	VAS tiredness (mean ± SD)	52.4 ± 33.5	45.5 ± 31.2	66.3 ± 35.5	ns
	VAS pain (mean ± SD)	49.1 ± 33.9	38.1 ± 31.6	71.0 ± 28.5	p=0.03
	VAS GH Patient (mean ± SD)	46.8 ± 32.6	39.6 ± 29.9	61.3 ± 35.1	ns
	VAS GH Physician (mean ± SD)	39.4 ± 23.8	31.8 ± 21.7	54.8 ± 21.2	ns
	LIKERT Patient (mean ± SD)	3.2 ± 1.1	2.8 ± 1.0	3.9 ± 1.0	p=0.03
	LIKERT Physician (mean ± SD)	2.7 ± 1.0	2.4 ± 1.1	3.3 ± 0.7	ns
	DAS (mean ± SD)	2.9 ± 1.5	2.5 ± 1.5	3.9 ± 1.0	p=0.03

B

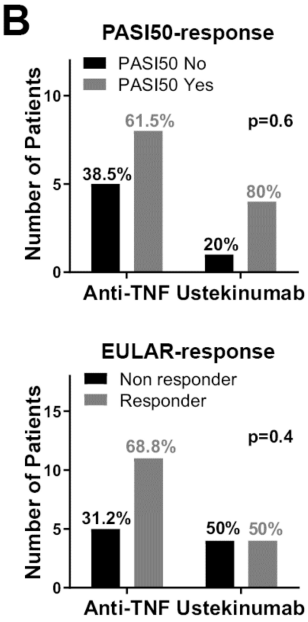


Figure S1 24-weeks characteristics of the patients included in the Psoriatic Arthritis Pathobiology and its Relationship with Clinical Disease Activity (PsABRE) study. A, Patients' characteristics at 24-weeks post-treatment (n=24, three patients lost to follow-up) and comparison between TNFi- (n=16) and ustekinumab-treated patients (n=8). p-values calculated using Mann–Whitney U-test or Fisher's exact test as required (TNFi-arm vs ustekinumab-arm). B, Skin (PASI50) and joints [EULAR(DAS) good/moderate vs none] response at 24-weeks. p-values calculated using Fisher's exact test.

SD, Standard Deviation; n, number; ESR, erythrocyte sedimentation rate; CRP, C-Reactive Protein; TJC, Tender Joints Count; SJC, Swollen Joints Count; RAI, Ritchie Articular Index; PASI, Psoriasis Area and Severity Index; VAS, Visual Analogue Scale (0-100); GH, Global Health; DAS, Disease Activity Score; TNF, Tumour Necrosis Factor; DMARDs, Disease Modifying Anti-Rheumatic Drugs; ns, non-significant; EULAR, European League Against Rheumatism.

Supplementary Table S1

		EULAR-RESPONSE		PASI50-RESPONSE	
PATIENT ID	TREATMENT	16 WEEKS	24 WEEKS	16 WEEKS	24 WEEKS
PsABRE-01	Anti-TNF	1	1	0	0
PsABRE-02	Anti-TNF	0	0	N/A	NA
PsABRE-03	Anti-TNF	0	LtFU	1	LtFU
PsABRE-04	Anti-TNF	1	1	0	0
PsABRE-05	Anti-TNF	1	1	N/A	NA
PsABRE-06	Anti-TNF	1	1	1	1
PsABRE-07	Anti-TNF	1	1	1	1
PsABRE-08	Anti-TNF	LtFU	LtFU	N/A	LtFU
PsABRE-09	Anti-TNF	1	1	0	1
PsABRE-10	Anti-TNF	0	0	1	1
PsABRE-11	Anti-TNF	1	1	0	0
PsABRE-12	Anti-TNF	1	1	1	1
PsABRE-13	Anti-TNF	0	0	1	1
PsABRE-14	Anti-TNF	1	1	0	0
PsABRE-15	Anti-TNF	1	1	1	1
PsABRE-16	Anti-TNF	1	1	0	1
PsABRE-17	Anti-TNF	1	0	0	0
PsABRE-18	Anti-TNF	0	0	N/A	NA
PsABRE-19	Ustekinumab	1	LtFU	0	LtFU
PsABRE-20	Ustekinumab	0	0	N/A	NA
PsABRE-21	Ustekinumab	0	1	1	1
PsABRE-22	Ustekinumab	0	1	1	1
PsABRE-23	Ustekinumab	1	1	1	1
PsABRE-24	Ustekinumab	0	1	N/A	NA
PsABRE-25	Ustekinumab	0	0	1	0
PsABRE-26	Ustekinumab	0	0	1	1
PsABRE-27	Ustekinumab	0	0	N/A	NA

Table S1 Summary of patients' treatment distribution (anti-TNF or ustekinumab), and individual joint [EULAR(DAS) good/moderate *versus* none] and skin (PASI50) responses.

0*, non-responder;* 1*, responder;* LtFU*, Lost to Follow-Up;* N/A*, Not Applicable;* TNF*, Tumour Necrosis Factor;* EULAR*, European League Against Rheumatism;* PASI*, Psoriasis Area and Severity Index.*

Supplementary Table S2

GENE NAME	TAQMAN PROBE	GENE NAME	TAQMAN PROBE
ANGPT1	Hs00919202_m1	*IL17D*	Hs00972161_m1
ANGPT2	Hs00169867_m1	*IL17F*	Hs01028648_m1
CCL1	Hs00234140_m1	*IL17RA*	Hs01056316_m1
CCL17	Hs00171074_m1	*IL17RB*	Hs00218889_m1
CCL19	Hs00171149_m1	*IL18*	Hs01038788_m1
CCL2	Hs00234140_m1	*IL1B*	Hs01555410_m1
CCL20	Hs00355476_m1	*IL1F10*	Hs00544661_m1
CCL21	Hs00171076_m1	*IL1R1*	Hs00991010_m1
CCL22	Hs01574247_m1	*IL2*	Hs00174114_m1
CCR2	Hs01560352_m1	*IL21*	Hs00222327_m1
CCR6	Hs00171121_m1	*IL22*	Hs01574154_m1
CD163	Hs00174705_m1	*IL23A*	Hs00372324_m1
CD28	Hs01007422_m1	*IL23R*	Hs00332759_m1
CD34	Hs02576480_m1	*IL36A*	Hs00205367_m1
CD40LG	Hs00163934_m1	*IL36B*	Hs00758166_m1
CD68	Hs00154355_m1	*IL36G*	Hs00219742_m1
CD8A	Hs00233520_m1	*IL36RN*	Hs00202179_m1
CSF1	Hs00174164_m1	*IL6*	Hs00174131_m1
CSF2	Hs99999044_m1	*IRF4*	Hs00180031_m1
CSF3	Hs99999083_m1	*MMP1*	Hs00899658_m1
CXCL12	Hs02829207_m1	*MMP13*	Hs00942584_m1
CXCL13	Hs00757930_m1	*MMP3*	Hs00968305_m1
CXCL2	Hs00601975_m1	*MMP9*	Hs00957562_m1
CXCL3	Hs00171061_m1	*MS4A1*	Hs00544819_m1
CXCL8	Hs00174103_m1	*PDPN*	Hs00366766_m1
CXCR4	Hs00976734_m1	*SOCS1*	Hs00705164_s1
CXCR5	Hs00173527_m1	*SOCS3*	Hs02330328_s1
DKK1	Hs00183740_m1	*STAT3*	Hs00374280_m1
F3	Hs01076029_m1	*STAT5A*	Hs00234181_m1
FOXP3	Hs01085834_m1	*STAT6*	Hs00598625_m1
GATA3	Hs00231122_m1	*TGFB1*	Hs00998133_m1
ICAM1	Hs00164932_m1	*TIMP1*	Hs01092511_m1
IFNG	Hs00989291_m1	*TIMP2*	Hs00234278_m1
IL10	Hs00961622_m1	*TLR4*	Hs00152939_m1
IL12A	Hs01073447_m1	*TNF*	Hs00174128_m1
IL12B	Hs01011518_m1	*TNFRSF11A*	Hs00921372_m1
IL12RB1	Hs01106578_m1	*TNFSF11*	Hs00243522_m1
IL15	Hs01003716_m1	*VCAM1*	Hs01003372_m1
IL17A	Hs00174383_m1	*VEGFA*	Hs00900055_m1
IL17C	Hs00171163_m1	*VEGFC*	Hs01099203_m1

Table S2 List of TaqMan probes used for gene expression analysis by real-time polymerase chain reaction (PCR) with Fluidigm technology (Fluidigm corporation, San Francisco, CA-USA).

Supplementary Table S3

		LOW INFLAMMATORY SCORE 0-1 (9 PATIENTS)	HIGH SYNOVIAL INFLAMMATORY SCORE 2-7 (15 PATIENTS)	p-values
BASELINE	Female % (n)	33.3% (3)	73.3% (11)	ns
	Age years (mean ± SD)	42.7 ± 12.9	48.1 ± 13.0	ns
	Disease duration years (mean ± SD)	13.3 ± 12.9	8.4 ± 12.0	ns
	ESR mm/hr (mean ± SD)	19.9 ± 17.3	27.5 ± 17.2	ns
	CRP mg/l mean (mean ± SD)	10.3 ± 18.8	14.0 ± 24.4	ns
	TJC/78 (mean ± SD)	34.8 ± 19.5	32.9 ± 23.6	ns
	TJC/68 (mean ± SD)	31.2 ± 16.3	29.9 ± 20.1	ns
	RAI (mean ± SD)	18.8 ± 10.4	18.3 ± 8.8	ns
	SJC/76 (mean ± SD)	10.8 ± 7.3	14.2 ± 11.1	ns
	SJC/66 (mean ± SD)	10.6 ± 7.4	13.7 ± 11.5	ns
	SJC/44 (mean ± SD)	9.1 ± 6.4	11.1 ± 9.8	ns
	Skin involvment, yes % (n)	77.7% (7)	73.3% (11)	ns
	PASI (mean ± SD)	5.7 ± 6.2	4.0 ± 3.7	ns
	VAS tiredness (mean ± SD)	58.1 ± 29.0	62.4 ± 29.8	ns
	VAS pain (mean ± SD)	63.0 ± 17.7	73.2 ± 23.7	ns
	VAS GH Patient (mean ± SD)	66.3 ± 18.9	71.7 ± 27.1	ns
	VAS GH Physician (mean ± SD)	58.2 ± 14.4	65.5 ± 12.1	ns
	LIKERT Patient (mean ± SD)	3.8 ± 0.7	3.8 ± 0.8	ns
	LIKERT Physician (mean ± SD)	3.3 ± 0.5	3.7 ± 0.6	ns
	DAS (mean ± SD)	4.1 ± 1.1	4.5 ± 1.2	ns
	HAQ (mean ± SD)	1.8 ± 0.6	2.0 ± 0.6	ns
	Previous anti-TNF, yes % (n)	33.3% (3)	33.3% (5)	ns
	Current DMARDs, yes % (n)	55.6% (5)	73.3% (11)	ns
	Assigned to Anti-TNF, % (n)	66.7% (6)	66.7% (10)	ns
	Assigned to Ustekinumab,% (n)	33.3% (3)	33.3% (5)	ns

Table S3 Baseline clinical variables comparison between patients characterized by a lower (n=9) or higher (n=15) synovial inflammatory score [13]. 24/27 baseline synovial tissues were included in the analysis; 3 samples were classified as “ungraded” (absence of lining layer and/or necrotic tissue and/or absent of macrophages in the sublining). p-values were calculated using Mann–Whitney U-test or Fisher's exact test as appropriate.

SD*, Standard Deviation;* n*, number*; ESR*, erythrocyte sedimentation rate;* CRP*, C-Reactive Protein;* TJC*, Tender Joints Count;* SJC*, Swollen Joints Count;* RAI*, Ritchie Articular Index;* PASI*, Psoriasis Area and Severity Index;* VAS*, Visual Analogue Scale (0-100);* GH*, Global Health;* DAS*, Disease Activity Score;* HAQ*, Health Assessment Questionnaire;* TNF*, Tumour Necrosis Factor;* DMARDs*, Disease Modifying Anti-Rheumatic Drugs;* ns*, non-significant.*

Supplementary Table S4

		LYMPHO-MYELOID (3 PATIENTS)	DIFFUSE-MYELOID (11 PATIENTS)	PAUCI-IMMMUNE (10 PATIENTS)	p-values
BASELINE	Female % (n)	66.7% (2)	72.7% (8)	40.0% (4)	ns
	Age years (mean ± SD)	52.1 ± 22.5	47.8 ± 11.0	42.4 ± 12.2	ns
	Disease duration years (mean ± SD)	22.7 ± 23.0	4.5 ± 2.1	12.5 ± 12.5	ns
	ESR mm/hr (mean ± SD)	23.7 ± 2.1	25.9 ± 17.9	23.6 ± 20.1	ns
	CRP mg/l mean (mean ± SD)	2.0 ± 3.5	17.1 ± 27.9	10.9 ± 17.8	ns
	TJC/78 (mean ± SD)	36.0 ± 31.2	28.9 ± 20.9	38.1 ± 21.2	ns
	TJC/68 (mean ± SD)	31.3 ± 26.6	27.0 ± 18.3	33.9 ± 17.5	ns
	RAI (mean ± SD)	20.7 ± 12.3	16.9 ± 8.3	19.5 ± 10.1	ns
	SJC/76 (mean ± SD)	15.7 ± 6.7	11.6 ± 9.8	13.6 ± 11.3	ns
	SJC/66 (mean ± SD)	14.7 ± 8.0	11.1 ± 10.0	13.4 ± 11.4	ns
	SJC/44 (mean ± SD)	9.7 ± 6.8	9.7 ± 9.2	11.3 ± 9.2	ns
	Skin involvment, yes % (n)	66.7% (2)	72.7% (8)	80.0% (8)	ns
	PASI (mean ± SD)	3.2 ± 4.0	3.8 ± 4.0	5.9 ± 5.7	ns
	VAS tiredness (mean ± SD)	61.7 ± 2.3	60.4 ± 34.3	61.0 ± 28.8	ns
	VAS pain (mean ± SD)	76.0 ± 3.6	70.0 ± 26.4	66.7 ± 20.4	ns
	VAS GH Patient (mean ± SD)	83.0 ± 10.8	67.0 ± 30.2	68.6 ± 19.2	ns
	VAS GH Physician (mean ± SD)	68.3 ± 12.1	63.7 ± 12.6	60.0 ± 14.7	ns
	LIKERT Patient (mean ± SD)	4.0 ± 1.0	3.6 ± 0.7	3.9 ± 0.7	ns
	LIKERT Physician (mean ± SD)	4.0 ± 0	3.6 ± 0.7	3.4 ± 0.5	ns
	DAS (mean ± SD)	4.6 ± 1.3	4.2 ± 1.1	4.4 ± 1.3	ns
	HAQ (mean ± SD)	2.4 ± 0.6	1.9 ± 0.6	1.7 ± 0.6	ns
	Previous anti-TNF, yes % (n)	33.3% (1)	27.3% (3)	40.0% (4)	ns
	Current DMARDs, yes % (n)	66.7% (2)	81.8% (9)	50.0% (5)	ns
	Assigned to Anti-TNF, % (n)	66.7% (2)	72.7% (8)	60.0% (6)	ns
	Assigned to Ustekinumab,% (n)	33.3% (1)	27.3% (3)	40.0% (4)	ns

Table S4 Baseline clinical variables comparison between patients characterized by a lympho-myeloid (n=3), diffuse-myeloid (n=11) or pauci-immune (n=10) pathotype as previously defined in rheumatoid arthritis [14]. Briefly, immune-cells infiltrate was defined by immunohistochemistry (CD3 for T-cells, CD20 for B-cells, CD68 for macrophages and CD138 for plasma cells) and quantified using a semi-quantitative score (0-4) [14]. Accordingly, patients were categorised as lympho-myeloid if CD20 score ≥2 and/or CD138 score >2, diffuse-myeloid if CD68 sublining score ≥2, CD20 score ≤1 and CD138 score ≤2, and pauci-immune if CD68 sublining score <2 and CD3/CD20/CD138 score <1. 24/27 baseline synovial tissues were included in the analysis; 3 samples were classified as "ungraded" (absence of lining layer and/or necrotic tissue and/or absent of macrophages in the sublining). p-values were calculated using Kruskal Wallis or Fisher's exact test as appropriate.

SD*, Standard Deviation;* n*, number*; ESR*, erythrocyte sedimentation rate;* CRP*, C-Reactive Protein;* TJC*, Tender Joints Count;* SJC*, Swollen Joints Count;* RAI*, Ritchie Articular Index;* PASI*, Psoriasis Area and Severity Index;* VAS*, Visual Analogue Scale (0-100);* GH*, Global Health;* DAS*, Disease Activity Score;* HAQ*, Health Assessment Questionnaire;* TNF*, Tumour Necrosis Factor;* DMARDs*, Disease Modifying Anti-Rheumatic Drugs;* ns*, non-significant.*

Supplementary Figure S2

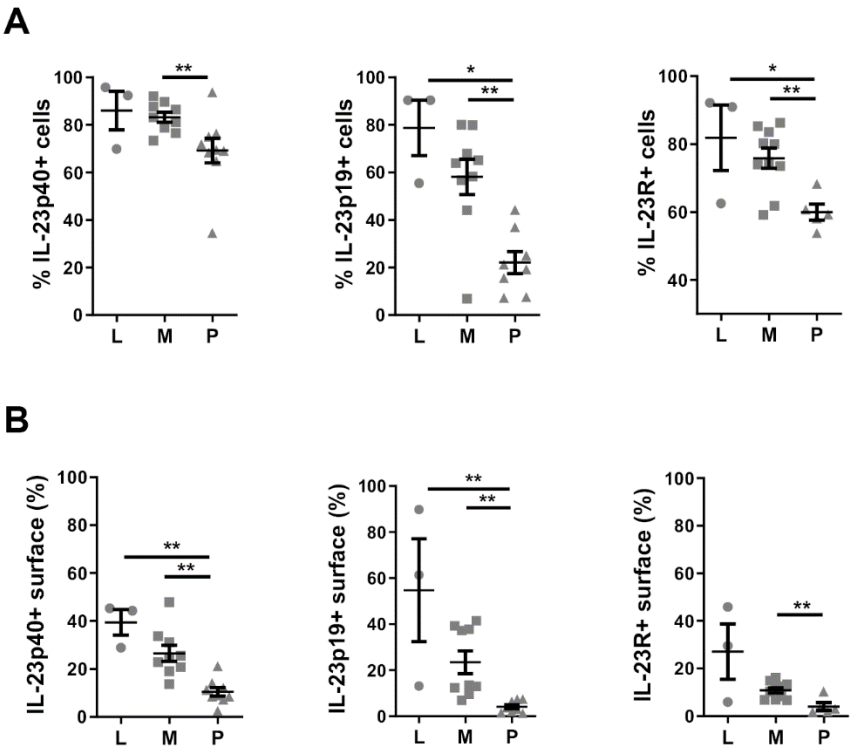


Figure S2 A, Distribution of the % of IL-23p40-, IL-23p19- and IL-23R-positive cells (of the total number of cells) according to the histological pathotypes at baseline. B, Distribution of the % of IL-23p40-, IL-23p19- and IL-23R-positive surface (of the total area) according to the histological pathotypes at baseline. L = **L**ympho-myeloid (n=3), M= diffuse-**M**yeloid (n=9-10), P= **P**auci-immune (n=5-9). Results are presented as mean ± standard deviation. *$p < 0.05$, **$p < 0.01$ as assessed by Kruskal–Wallis with Dunn's post-test.

Supplementary Table S5

	IL-23p40	IL-23p19	IL-23R
Anti-TNF	76.8 ± 14.0	43.2 ± 28.6	70.7 ± 14.2
Ustekinumab	78.0 ± 13.3	55.1 ± 27.8	72.1 ± 12.6

Table S5 IL-23p19, IL-23p40 and IL-23R baseline synovial protein expression levels stratified according to the biologic DMARD received afterwards (anti-TNF or ustekinumab). IL-23p19, IL-23p40 and IL-23R were detected by immunohistochemistry and quantified by Digital Image Analysis. Mean ± Standard Deviation of % of positive cells are represented. Anti-TNF: n=14-16; ustekinumab: n = 5-6.

Supplementary Table S6

		IL-23p40		IL-23p19		IL-23R	
		r	p-values	r	p-values	r	p-values
BASELINE	ESR mm/hr	0.04	0.84, ns	0.02	0.93, ns	0.39	0.10, ns
	CRP mg/l	0.02	0.92, ns	0.15	0.53, ns	0.14	0.57, ns
	TJC/78	-0.14	0.54, ns	-0.09	0.70, ns	-0.26	0.28, ns
	TJC/68	-0.13	0.56, ns	-0.09	0.70, ns	-0.28	0.25, ns
	RAI	-0.02	0.91, ns	0.02	0.95, ns	-0.05	0.83, ns
	SJC/76	0.10	0.67, ns	0.21	0.37, ns	-0.31	0.19, ns
	SJC/66	0.08	0.73, ns	0.11	0.65, ns	-0.34	0.16, ns
	SJC/44	0.05	0.83, ns	-0.05	0.83, ns	-0.44	0.06, ns
	PASI	-0.13	0.61, ns	0.16	0.57, ns	0.13	0.64, ns
	VAS tiredness	0.13	0.56, ns	0.31	0.19, ns	-0.17	0.48, ns
	VAS pain	-0.21	0.34, ns	0.32	0.17, ns	-0.32	0.18, ns
	VAS GH Patient	-0.09	0.70, ns	0.31	0.19, ns	-0.33	0.17, ns
	VAS GH Physician	0.12	0.61, ns	0.14	0.54, ns	-0.18	0.45, ns
	LIKERT Patient	-0.52	0.01, *	0.05	0.84, ns	-0.63	0.004, **
	LIKERT Physician	0.08	0.73, ns	0.44	0.05, ns	-0.08	0.73, ns
	DAS	0.13	0.57, ns	0.09	0.70, ns	-0.16	0.51, ns
	HAQ	0.29	0.20, ns	0.38	0.10, ns	0.13	0.58, ns

Table S6 Correlations between IL-23p40/IL-23p19/IL-23R protein expression (as percentage of positive cells assessed by IHC) and baseline clinical variables. p-values were calculated using Spearman r test. *p < 0.05, **p < 0.01

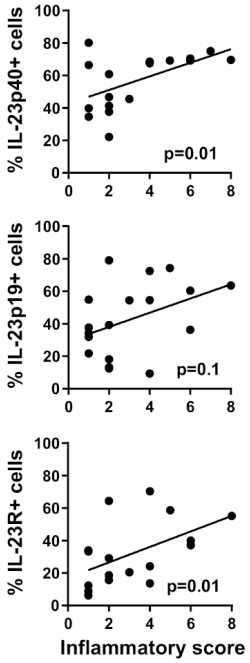
ESR*, erythrocyte sedimentation rate;* CRP*, C-Reactive Protein;* TJC*, Tender Joints Count;* SJC*, Swollen Joints Count;* RAI*, Ritchie Articular Index;* PASI*, Psoriasis Area and Severity Index;* VAS*, Visual Analogue Scale (0-100);* GH*, Global Health;* DAS*, Disease Activity Score;* HAQ*, Health Assessment Questionnaire;* TNF*, Tumour Necrosis Factor;* DMARDs*, Disease Modifying Anti-Rheumatic Drugs;* ns*, non-significant.*

Supplementary Figure S3

A

EARLY PsA	TOTAL POPULATION (21 PATIENTS)
Female % (n)	57.1% (12)
Age years (mean ± SD)	42.0 ± 14.4
Disease duration months (mean ± SD)	4.9 ± 2.7
ESR mm/hr (mean ± SD)	33.8 ± 21.8
CRP mg/l mean (mean ± SD)	19.4 ± 19.3
TJC/28 (mean ± SD)	7.8 ± 8.1
SJC/28 (mean ± SD)	4.4 ± 3.4
VAS tiredness (mean ± SD)	48.4 ± 28.4
VAS pain (mean ± SD)	54.3 ± 24.2
VAS GH Patient (mean ± SD)	63.0 ± 24.2
VAS GH Physician (mean ± SD)	54.3 ± 25.7
DAS/28 (mean ± SD)	4.7 ± 1.2
HAQ (mean ± SD)	1.3 ± 0.9

B



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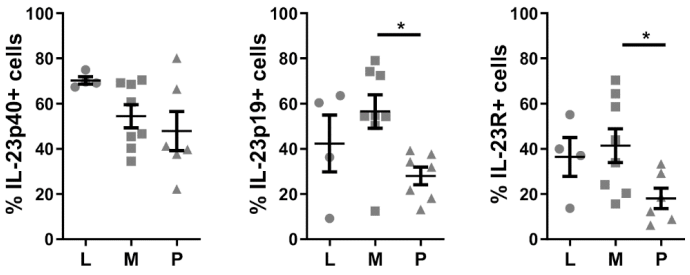


D

EARLY PsA	IL-23p40		IL-23p19		IL-23R	
	r	p-values	r	p-values	r	p-values
ESR mm/hr	0.22	0.37, ns	0.20	0.42, ns	-0.02	0.93, ns
CRP mg/l	0.23	0.37, ns	0.21	0.39, ns	-0.07	0.77, ns
TJC/28	-0.32	0.19, ns	-0.36	0.13, ns	0.04	0.87, ns
SJC/28	-0.04	0.88, ns	0.13	0.60, ns	0.32	0.2, ns
VAS tiredness	-0.09	0.72, ns	-0.16	0.51, ns	0.30	0.23, ns
VAS pain	0.10	0.69, ns	-0.24	0.31, ns	-0.10	0.69, ns
VAS GH Patient	-0.35	0.16, ns	-0.22	0.37, ns	-0.01	0.98, ns
VAS GH Physician	-0.08	0.76, ns	-0.24	0.33, ns	-0.11	0.65, ns
DAS /28	-0.15	0.55, ns	-0.31	0.20, ns	-0.03	0.9, ns
HAQ	-0.33	0.19, ns	-0.28	0.25, ns	-0.04	0.87, ns

Figure S3 IL-23 cytokines and receptor expression in early Psoriatic Arthritis. A, Demographic and clinical features of the early PsA cohort (n=21); B, Correlations between inflammatory scores and IL-23p40, IL-23p19 or IL-23R percentages of positive cells within the synovial tissue. p-values, calculated by Spearman's bivariate correlation analysis, are shown on each graph. C, Distribution of the % of IL-23p40-, IL-23p19- and IL-23R-positive cells (of the total number of cells) according to the histological pathotypes at baseline. L = **L**ympho-myeloid (n=4), M= diffuse-**M**yeloid (n=7-10), P= **P**auci-immune

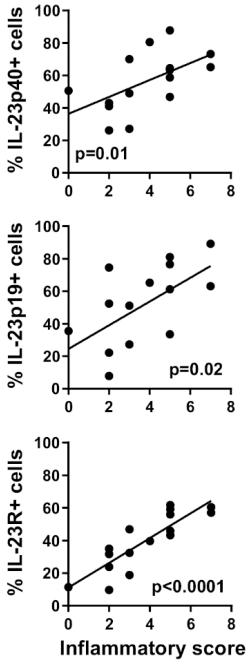
(n=6-7). Results are presented as mean ± standard deviation. *p < 0.05 as assessed by Kruskal–Wallis with Dunn's post-test. D, Correlations between IL-23p40/IL-23p19/IL-23R protein expression (as percentage of positive cells assessed by IHC) and baseline clinical variables. p-values were calculated using Spearman r test. *SD, Standard Deviation; n, number; ESR, erythrocyte sedimentation rate; CRP, C-Reactive Protein; TJC, Tender Joints Count; SJC, Swollen Joints Count; VAS, Visual Analogue Scale (0-100); GH, Global Health; DAS, Disease Activity Score; HAQ, Health Assessment Questionnaire; ns, non-significant.*

Supplementary Figure S4

A

	TOTAL POPULATION (17 PATIENTS)
EARLY RA	
Female % (n)	70.6% (12)
Age years (mean ± SD)	43.4 ± 14.8
Disease duration months (mean ± SD)	4.4 ± 2.4
ESR mm/hr (mean ± SD)	27.9 ± 20.4
CRP mg/l mean (mean ± SD)	11.9 ± 12.5
TJC/28 (mean ± SD)	9.7 ± 5.5
SJC/28 (mean ± SD)	5.9 ± 2.4
VAS tiredness (mean ± SD)	29.1 ± 24.4
VAS pain (mean ± SD)	46.1 ± 29.1
VAS GH Patient (mean ± SD)	61.5 ± 24.4
VAS GH Physician (mean ± SD)	51.2 ± 23.9
RF+, yes % (n)	76.5% (13)
CCP+, yes % (n)	70.6% (12)
DAS/28 (mean ± SD)	5.0 ± 0.9
HAQ (mean ± SD)	1.1 ± 0.6

B



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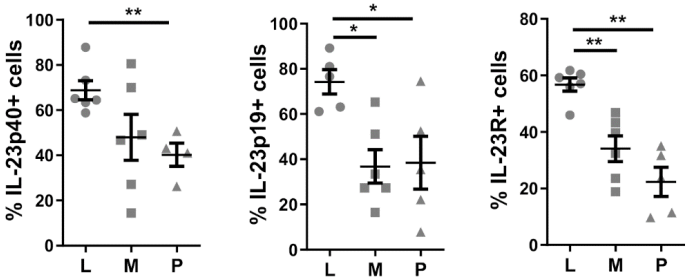

D

	IL-23p40		IL-23p19		IL-23R	
	r	p-values	r	p-values	r	p-values
EARLY RA						
ESR mm/hr	0.38	0.14, ns	0.34	0.19, ns	0.53	0.03, *
CRP mg/l	0.02	0.95, ns	0.17	0.52, ns	0.37	0.15, ns
TJC/28	0.40	0.13, ns	0.47	0.07, ns	0.28	0.27, ns
SJC/28	0.13	0.64, ns	0.32	0.23, ns	0.04	0.88, ns
VAS tiredness	0.39	0.13, ns	0.18	0.51, ns	0.29	0.25, ns
VAS pain	0.23	0.39, ns	0.63	0.01, *	0.46	0.07, ns
VAS GH Patient	0.06	0.83, ns	0.40	0.12, ns	0.30	0.24, ns
VAS GH Physician	0.24	0.36, ns	0.36	0.17, ns	0.27	0.29, ns
RF titer	0.06	0.83, ns	0.20	0.46, ns	0.01	0.97, ns
CCP titer	0.46	0.07, ns	0.34	0.19, ns	0.47	0.06, , ns
DAS /28	0.25	0.35, ns	0.51	0.05, *	0.33	0.19, , ns
HAQ	0.11	0.67, ns	0.22	0.41, ns	0.27	0.29, ns

Figure S4 IL-23 cytokines and receptor expression in early Rheumatoid Arthritis. A, Demographic and clinical features of the early arthritis RA cohort (n=17); B, Correlations between inflammatory scores and IL-23p40, IL-23p19 or IL-23R percentages of positive cells within the synovial tissue. p-values, calculated by Spearman's bivariate correlation analysis, are shown on each graph. C, Distribution of the % of IL-23p40-, IL-23p19- and IL-23R-positive cells (of the total number of cells) according to the histological pathotypes at baseline. L = **L**ympho-myeloid (n=6), M= diffuse-**M**yeloid (n=6), P= **P**auci-immune (n=5). Results are presented as mean ± standard deviation. *p < 0.05, **p < 0.01 as assessed

by Kruskal–Wallis with Dunn's post-test. D, Correlations between IL-23p40/IL-23p19/IL-23R protein expression (as percentage of positive cells assessed by IHC) and baseline clinical variables. *p < 0.05, p-values were calculated using Spearman r test. *SD, Standard Deviation; n, number; ESR, erythrocyte sedimentation rate; CRP, C-Reactive Protein; TJC, Tender Joints Count; SJC, Swollen Joints Count; VAS, Visual Analogue Scale (0-100); GH, Global Health; DAS, Disease Activity Score; HAQ, Health Assessment Questionnaire; ns, non-significant.*